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CONFERENCE CONTRIBUTION

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Reference materials for small-sample analysis

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Abstract Many modern analytical techniques use small solid samples and lack proper reference materials for their calibration and quality assurance. A remedy to this deficiency may be in the development of a new genre of highly homogeneous natural matrix materials, their properties being studied with analytical techniques such as PIXE and μ-PIXE, solid sampling AAS, scanning electron microscopy in combination with electron probe X-ray microanalysis, and INAA. Suitable natural materials may be obtained in form of single cell biological materials, finely dispersed suspensions and precipitates such as air particulate matter or sediments, and by appropriate particle size reduction of complex matrices. Initial studies have been carried out on single cell green algae biomass and air particulate matter, as well as several processed materials. Narrow particle size distributions with particles preferably below 10 μm diameter may assure the desired analytical homogeneity. The determination of sampling parameters for individual measurands will ascertain the utility of a material for small-sample analysis.

Introduction

As analytical techniques have become more sensitive, they require ever smaller samples for the determination of natural and pollutant elements that are investigated in biological and environmental materials. Concurrent with this development, new procedures are employed using solid samples for the analysis, rather than dissolutions of samples, to determine either concentrations in the bulk sample or the distribution of elements in smaller components of the bulk sample. These techniques not only include those that through their physical principles predominantly characterize solid samples, such as X-ray fluorescence, proton induced X-ray emission (including their application in microprobes), and also neutron activation analysis; but also many other techniques that were developed using sample dissolutions and now have capabilities for solid sample introduction, including atomic absorption, and inductively-coupled plasma optical emission and mass spectrometry. Other probe techniques, such as spark source and laser ablation mass spectrometry, electron- and ion-microprobe X-ray emission spectrometry, etc., may also find applications in biological and environmental studies. Table 1 presents an overview of the most widely used solid sample techniques and the typical sample sizes analyzed or consumed in the determinations.

Unfortunately, this wide variety of effective techniques is confronted with two major problems: Many of these techniques are affected in their accuracy by the matrix composition, i.e., standards for calibration and quality assurance must match the analyzed samples as closely as possible, and essentially no natural matrix certified reference materials (CRMs) have been produced for small-sample analysis. Direct application of most existing CRMs in solid sampling procedures, i.e., analyses of samples having masses considerably smaller than 100 mg, more typically ≤ 1 mg, is often difficult or even impossible because trace components may not be sufficiently homogeneously distributed in the samples or their homogeneous distribution has not been tested. A recent survey on biological and environmental CRMs revealed that the smallest recommended samples sizes are 100 mg [1]. However, CRMs made of artificial composites, such as alloys and glasses can be homogeneous at smaller sample sizes and are used to some degree by the techniques discussed.

Homogeneity

Homogeneity is considered to be the most vital prerequisite for a CRM; more stringent homogeneity requirements exist for the analysis of small subsamples. Environmental CRMs, and also plant and tissue materials, typically con-

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Table 1 Overview of solid sampling techniques with typical sample sizes analyzed or consumed

Solid sampling technique	Typical sample mass
Neutron Activation Analysis (INAA)	1 mg – 10 g
X-Ray Fluorescence (XRF)	1 – 100 mg
Particle-Induced X-Ray Emission (PIXE)	0.1 – 10 mg
Particle-Induced Nuclear Reaction Analysis (PIGE)	0.1 – 10 mg
Rutherford Backscattering Spectrometry (RBS)	0.1 – 10 mg
Spark Source Mass Spectrometry (SSMS)	0.1 – 10 mg
Solid Sampling	
Atomic Absorption Spectrometry (SS-ZAAS)	0.1 – 1 mg
Inductively Coupled Optical Emission Spectrometry (SS-ICPOES)	0.1 – 1 mg
Inductively Coupled Mass Spectrometry (SS-ICPMS)	0.1 – 1 mg
Glow Discharge Mass Spectrometry (GDMS)	1 – 100 µg
Laser Ablation	
Inductively Coupled Optical Emission Spectrometry	1 – 10 ng
Inductively Coupled Mass Spectrometry	1 – 10 ng
Micro-PIXE	down to 0.1 ng
Micro-Synchrotron Induced XRF	down to 0.1 ng
Electron Microprobe Analysis (EMPA)	down to 1 pg
Laser Microprobe Analysis (LAMMA)	down to 1 pg
Secondary Ion Mass Analysis (SIMS)	down to 1 pg
Transmission Electron Microscopy with Energy Dispersive X-Ray Analysis (EPXMA)	down to 1 fg

sist of several different solid phases which have characteristic physical properties (e.g. grain size, particle geometry, density etc.) in addition to widely varying concentrations of trace elements in these different solid phases.

The notion of homogeneity of a material is not an absolute one and should be discussed taking into account factors such as the type of material, the sample size for the typical analysis, the kind of species (measurands), and the requirements of the user and the applied analytical technique. For natural matrix CRMs, which in most cases are inhomogeneous on a microscopic scale, homogeneity means that subsamples of a material have the same average composition within appropriately chosen confidence limits. Alternatively, homogeneity is achieved when replicate subsamples of the size (mass) required by the trace analytical or microanalytical technique do not show significant differences within the precision limits of the method. In most instances, this is assured by providing a sufficiently large sum of similar mass entities (particles) for the individual test.

Experience has shown that micro-heterogeneities (of otherwise homogeneous samples), found at low sample masses, may cause serious problems. This is usually the consequence of larger particles (referred to by some authors as "nuggets") which can have much higher trace element concentrations and thus bear little resemblance to the bulk composition of the sample. In the case of "nuggets", the degree of homogeneity cannot be directly estimated or calculated from the variance of analytical data, and in such cases statistical evaluations using a Poisson-type probability function is necessary to obtain realistic values for homogeneity [2].

Particle sizes in natural matrix CRMs

Many of the natural matrix CRMs are prepared from bulk samples by grinding and milling them to a certain particle size, which is expected to provide a more homogeneous material. Techniques for material preparation include conventional mechanical mills, cryogenic milling and air-jet milling. Many of the preparations are accompanied by a size classification and exclusion through sieving or cyclone separation. Depending on these approaches, the typical particle sizes in natural matrix CRMs range from several µm to hundreds of µm with some CRMs having even larger particles [3]. Considering the current recommended sample sizes for most biological and environmental reference materials being more than 100 mg, natural materials that are prepared to a smaller particle size or occur at a small particle size, the best condition would be a narrow size distribution of particles not larger than 10 µm, probably will fulfill the requirements for homogeneity at smaller sample sizes.

A number of recent CRMs were produced to near ideal particle size distributions by air-jet milling, among others are NIST SRM 1570a, Spinach and IAEA-375, Cabbage [4]. Figure 1 shows that the latter two materials have a

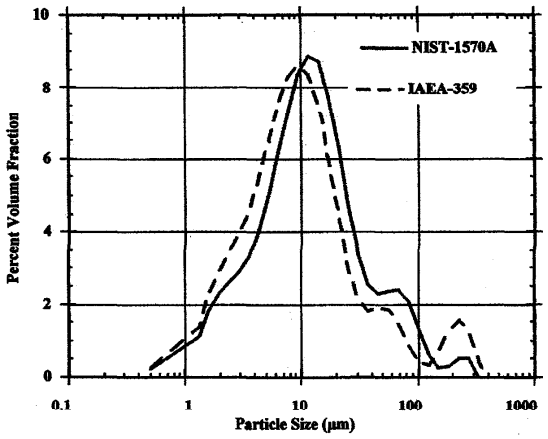

Fig. 1 Particle size of two air-jet milled biological materials, NIST SRM 1570a, Spinach, and IAEA-359, Cabbage

peak particle size of about 10 µm and form fine, free flowing powders. These should be very homogeneous; however, their homogeneity at small sample sizes has not been systematically evaluated. Other natural reference materials have not been produced to similar small particle sizes and distributions when, for example, only conventional milling and sieving is employed to arrive at a more homogeneous material.

Since the milling of materials is costly and has some drawbacks, such as possible contamination and limited success in providing small and rather homogeneous size particles, as well as loss of significant amounts of material during such preparations, natural materials that already occur as small particles such as air particulate matter, certain sediments, and cellular biological materials may form the basis of the required reference materials. To explore this approach, the IAEA Analytical Quality Control Services (AQCS) has introduced a single cell algae and an air particulate matter candidate CRM for studies in a Coordinated Research Program on reference materials for microanalytical nuclear techniques [5]. The nature of these materials, i.e., naturally occurring particles, may provide ideal model reference material for the techniques discussed.

Determination of homogeneity

From the above discussion on homogeneity, it follows that materials that consist of particles of widely different composition can be made apparently homogeneous for certain measurands and measurement techniques by appropriate grinding and mixing or are homogeneous by the particulate nature of the material. Therefore, the determination of homogeneity of natural matrix materials will involve the statistical evaluation of measurement data under consideration of the particulate nature of the materials.

In choosing appropriate measurement techniques, the following criteria should be met: i) the technique ideally is nondestructive or consumes the sample as is without preparation, ii) the technique should involve small and well-defined uncertainties (measurement imprecision of less than 2% may be necessary) and iii) should be capable to efficiently determine the composition of statistically relevant numbers of samples. Techniques like EPXMA, micro-PIXE, INAA and SS-ZAAS have proved to be suitable for homogeneity investigations due to their inherent low sample mass requirements. These techniques can provide information on composition of microgram or even nanogram levels of sample masses (i.e., comparable in many instances to the mass of individual particles).

Most commonly quantitative estimates of homogeneity are made by calculating from the appropriate analytical data a sampling constant according to Ingamells [6]:

$$K_S = R^2 \cdot w \tag{1}$$

where K_S = sampling constant; R = relative standard deviation; and w = sample mass.

This constant was originally presented for use in geological sampling with considerably larger amounts of sample used in analysis than for the techniques discussed here and therefore often results in the estimation of rather large sample sizes (the defined value of the sampling constant for a measurand of interest) that would provide no more than one percent uncertainty due to inhomogeneity. For smaller samples, the convention by Kurfürst that defines for each measurand a relative homogeneity factor H_E, based on the principles of Ingamells, appears to be more suitable [7]:

$$H_E = R_{HOM} \cdot \sqrt{w} \tag{2}$$

and

$$R^2 = R^2_{HOM} + R^2_{AN} \tag{2.1}$$

where

H_E = the homogeneity factor.

Homogeneity factors of $H_E < 10$ determined in the small sample masses used e.g. in SS-ZAAS are considered sufficient for the material's homogeneity, especially when a large number of elements has similar homogeneity factors. For the accurate estimate of either one, the Ingamells sampling constant (Eq. 1) and the Kurfürst homogeneity factor (Eq. 2), it is mandatory to establish the analytical uncertainty budget for the measurements from which the quantitative extraction of the uncertainty due to inhomogeneity may be possible (Eq. 2.1). Very precise analytical measurements made, if possible, at significantly different sample sizes, will aid the correct estimation of a material's homogeneity.

Conclusions

A number of naturally suitable or appropriately prepared environmental and biological CRMs can be identified as candidate materials for the quality assurance needs for small-sample techniques. Indications for their homogene-

ity can be derived from their particle sizes and distributions [3], as well as from analytical data. Sampling constants for IAEA-390 single cell algal biomass samples [8] and IAEA-396A/M urban particulate matter [9] have been determined in the milligram range and homogeneity factors in the same material were consistent and smaller than 10 for a number of elements [5]. It is expected that several more of the existing CRMs are of similar quality when studied for their analytical properties at small sample sizes. A moderate effort by the issuing organizations may make them available to the analytical community. In the end, particle size distributions and sampling constants or homogeneity factors should be made part of the information provided to users of CRMs.

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